CANADA/U.S. SPRUCE BUDWORM PROGRAM - WEST

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Title: Field evaluation of *Bacillus thuringiensis* for control of the western spruce budworm: Aerial application trials

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SUMMARY

The effectiveness of three formulations of *Bacillus thuringiensis*, Thuricide-16B, Thuricide-32B, and Dipel 4L, were compared at a dosage of 8 BIU/gal per acre against the western spruce budworm, *Choristoneura occidentalis*, at the Kaibab National Forest, Arizona. The treatments and untreated check were replicated 5 times on plots of 80 acres in size. A Marsh turbo thrush equipped with a standard spray boom and 28 No. 8-W fulljet nozzles was used to apply the treatments between the hours of 5-9 am over the period from June 25 to 28. Over 80% of the budworm larvae had reached the 5th instar before the completion of spraying.

Budworm mortality averaged approximately 90% on the treated plots at 26 days following treatment, with no difference is rates among the three products. However, the reduction was significantly greater than on the check plots that averaged 80%. The high rate of check mortality was largely the result of starvation. Many trees had been completely defoliated more than one week before the final population density sample. Budworm parasites were not adversly affected by Bt.

No evidence was found to indicate a spread of Bt infection by contagion in the budworm population after spraying. Infection rates dropped sharply from a maximum of 40% at 5 days to less than 10% at 12 days and 2% at 26 days, of the larvae collected from population density samples and reared on diet. In other studies, a bioassay showed that more than 50% of the original activity of spray residues on the foliage was lost within 3 days and 97.8% within 5 days after spraying.

Foliage protection was adversly affected by the delay in spray application. All of the Bt treatments showed a trend toward foliage protection, however, only the Thuricide treatments were significantly different from the check and then at $P \leq 10\%$.

INTRODUCTION

The western spruce budworm, Choristoneura occidentalis Freeman, is the most destructive defoliator of mixed conifer forests in western North America (Carolin and Honing 1972). New foliage of Douglas-fir, grand fir, white fir, subalpine fir, blue spruce, Engelmann spruce, and white spruce is preferred by this pest. Outbreaks of budworm are often characterized by high population densities and extensive feeding damage to current growth over prolonged periods of 3 to 5 years in duration.

Commercial formulations of microbial pesticides containing the bacterium, Bacillus thuringiensis Berliner (B.t.), has been used successfully for control of a variety of lepidopterous pests (Harper 1974, Hanson et al. 1975, Dunbar et al. 1973, Dimond et al. 1980). Tripp (1972) reported population reductions of 96 to 99 percent following an aerial application of B.t. against the spruce budworm, Choristoneura fumiferana, on balsamfir. Good foliage protection was reported by Smirnoff et al. (1976) and Smirnoff (1974) following applications of B.t. combined with the enzyme chitinase. Field efficacy of B.t. was increased by the addition of small quantities of Orthene against moderate budworm densities but did not provide satisfactory control of high populations (Morris 1977). Efficacy trials using B.t. against the budworm in western United States and Canada have generally shown unacceptable control of larval populations but favorable control of feeding damage (McGregor et al. 1976, Thompson et al. 1977, Hodgkinson et al. 1979).

Because _Bacillis thuringiensis_ has been shown to be completely harmless to man and other nontarget organisms, its development as a control agent against budworms can provide land managers with a valuable alternative to chemical pesticides.

In 1980, a series of coordinated trials to evaluate the effectiveness of B.t. against spruce budworms were conducted at several geographical areas in the U.S. and Canada. The overall test objectives were to compare two commercial formulations applied in as uniform a manner as possible at the various locations. Specific objectives of the test were to: (1) measure postspray reductions in larval populations; (2) estimate the degree of foliage protection provided by the treatments; (3) determine the postspray residual life of B.t. spray deposits on the foliage; and (4) measure the effects of the B.t. treatments on budworm parasites.

MATERIALS AND METHODS

Treatments

The following treatments were selected for comparison in the field efficacy trials: (1) Thuricide-16B, (2) Thuricide-32B, and (3) Dipel 4L. All of the materials were diluted in water to a final concentration that contained 8 billion international units (BIU)/gal for application at the rate of 1 gpa. The Thuricide-32B tank mix contained 25 percent molasses (vol./vol.). Chevron sticker at 1 gal/100 gal, and Rhodamine B dye at 1 lb/100 gal was included in all tank mixes. Each of the 3 B.t. treatments and an untreated control were assigned completely at random to 5 plots (reps). The plots were 80 acres (32.4 ha) in size.

Application

A Marsh turbo thrush fitted with a standard boom and 28 No. 8 fulljet nozzle tips was used to apply the treatments. The aircraft flew at 50 to 75 ft above the canopy at 150 mph on 100-foot swath widths. Plans to time the applications against peak fourth-stage larvae were aborted due to an accident involving the spray contractor. Due to the delay, over 80 percent of the budworm population had entered the fifth stage by the second day of spraying. Only one plot was sprayed on the first day (June 25) when the applicator couldn't locate the plot boundaries, and a fixed-wing aircract to provide guidance was ineffective. Arrival of a helicopter to mark swaths proved very effective, and we were able to spray 3, 5, and 6 plots on each of the following 3 days.

Study Location

Tests were conducted on the Kaibab NF between Jacob Lake and North Rim Grand Canyon, Arizona. The study plots were all located within a 10-mile radium of the Kaibab Lodge, at elevations ranging from 8,600 to 8,900 ft. Plots were separated by at least 0.5 miles to minimize possible spray drift between plots during application. The mixed conifer type contained varying proportions of Douglas-fir, white fir, and Engelmann spruce.

Sample Trees

Preference was given to using Douglas-fir trees for the efficacy measurements on the plots, but in some areas, white fir and Engelmann spruce trees had to be utilized to meet sample size requirements. However, in making response calculations, no attempt was made to stratify the data based on host species. A total of 30 trees were selected for population sampling on each plot. The trees were open-grown, fully crowned trees 30 to 50 feet tall. The sample trees were distributed along a line perpendicular to the flight path of the spray aircraft and near the middle of the plot.

Population-Density Samples

Spruce budworm larval population densities were determined at 24 to 48 hours prespray, and at 5, 12, 19, and 26 days postspray, using a sampling procedure described by Carolin and Coulter (1972). A pole pruner fitted with a basket was used to clip a 38-cm branch sample from midcrown. A total of 30 branch samples/plot were collected at all sample intervals except the 26-day period when 60 samples were taken. The branches were placed in 1/6 barrel-sized paper bags, stapled shut, and taken to the field laboratory for processing on the same day. The samples were examined to measure the length and width of the branches, and to count and record the number of budworm larvae and current shoots (buds) present. Density was expressed as the number of budworm/0.64 m^2 branch area (1,000 in^2).

Defoliation Estimates

Defoliation estimates were based on an examination of one midcrown branch collected from each of the 30 sample trees on the plots at the time of the 26-day population evaluation. Approximately 90 percent of the budworm population had pupated or the adult moths had already emerged
1979 and 1980 growth on each of the first
the right
at this time. On each branch, the outer 25 shoots on one side of the
rated
branch were classified as to the amount of ~~current~~ needles missing or destroyed within six class limits: 0-10, 11-25, 26-50, 51-75, 76-90, and 91-100 percent.

Larval Rearings

Budworm larvae on the branch samples provided material for larval rearing studies. Up to 10 larvae per branch were collected individually into disposable plastic petri dishes (No. 1006, Falcon Plastics) containing artificial insect diet and held until death or pupation. These collections were taken from all plots at the prespray and all of the postspray sample intervals. Results of these rearings provided the data from which rates of parasitism, B.t. mortality, and infection by virus were calculated.

Physical Spray Deposit Assessment

Immediately before spraying, a kromekote spray deposit card and millipore filter were placed at each of 30 stations distributed across the spray plots on a road near the center of the plot and perpendicular to the flight path. The kromekote cards are used to estimate droplet density, dropsize spectra (vmd), and gpa. Millipore filters were sent to N. Dubois (Hamden, CT) for culture and an estimate of drop density.

Biological Spray Deposit Assessment

An attempt was made to estimate the persistence of B.t. spray residues on the foliage following aerial application of the B.t. treatments. Portions of current shoots were clipped from each of two midcrown branches from 15 trees/plot at 2 hours, 3, and 5 days after spraying. The shoots were used to provision 7-oz rearing containers and then stocked with 10 fourth-stage budworm larvae taken from a disease-free laboratory culture. This provided a total of 15 cups from five replicates per treatment. Similar rearings were established on foliage samples taken from the untreated control plots. Mortality counts and diagnoses for B.t. were taken after 14 days in rearing.

RESULTS AND DISCUSSION

Comprehensive larval collections and instar determinations on June 18 showed about 80 percent of the budworm population to be in the second and third stages of development. A decision was made to begin spraying on June 23 although only partial budburst was noted on white fir and Douglas-fir and no budburst on Engelmann spruce at this time. An accident involving the contractor resulted in a delay in spray operations to June 25, and then only one plot was treated. Head-capsule measurements on June 26 showed about 80 percent of the larvae already in the fifth and sixth stages, and severe feeding damage was noticeable on several of the plots. These circumstances must be considered to have had an unfavorable effect on efficacy of the treatments.

Population Control

Spruce budworm population densities, averaged over all five plots for each treatment, are presented in table 1. Prespray densities ranged from 158.6 to 212.1 larvae/0.64 m^2 (1,000 in^2) of foliage. At 26 days, budworm densities had dropped to less than 20 in the B.t. treatments as compared to 35 in the control. Postspray budworm survival rates were calculated from the data in table 1 and are plotted in figure 1. These data were analyzed as for a CRD, with the four postspray times handled as split-plot treatments. Survival rates were significantly different, averaged over all times, and reflect the lower survival of budworm on the B.t.-treated areas. Also, the periods differed, and they were linearly related to time. There was no interaction of the percent larval survival with time which indicates that the treatment responses, in relationship to each other, remained approximately constant. Tukey's multiple-comparison test showed the survival rates in the three B.t. treatments were significantly lower than the control but not from each other (05 level).

The corrected population reductions on the treated plots at 26 days (44.4, 59.2, and 48.5 percent for Thuricide-16B, Thuricide-32B, and Dipel, respectively) are comparable to the results of tests of B.t. against the budworm in Montana (McGregor et al. 1976) Washington (Thompson et al. 1970) and British Columbia (Hodgkinson et al. 1979). Applications of B.t. on the same plot over 3 consecutive years in Quebec resulted in annual population reductions of 62.0, 59.8, and 54.9 percent (Smirnoff 1980). These results support the conclusion that under current application strategies, i.e., one application of 8 BIU/gal per acre will control 30 to 70 percent of the budworm population.

Residual Activity of Spray Deposits

The activity of the spray deposits on the plots treated with the three B.t. products declined rapidly with time (table 2), with differences between intervals significant at the 1-percent level. Mortality of budworm larvae fed foliage collected within 2 hours after spraying averaged 32.1 percent, over all treatments. Initial activity in the Thuricide treatments was almost 2 times greater than with Dipel and would appear to be related to the reduced spray deposits on Kromekote cards for the Dipel treatment (table 3). More than 50 percent of the original activity had been lost at 3 days, and only slight B.t. infection occurred among larvae fed foliage collected at 5 days. Neisess (1979) reported that molasses formulations are much less stable than water formulations of B.t. in rain. This would explain the more rapid loss of activity of the Thuricide-32B at 3 days since more than 0.5 in. of rainfall occurred on the second day after spraying.

Larval Rearing

During the summer, a total of 9,644 budworm larvae were collected from the population-density branch samples and reared in individual petri dishes. These rearings provided a measure of the prevalence of disease and parasites in the population. In general, parasite activity was not affected by the B.t. treatments (table 4). Rates of parasitism were the same at all timings, except for a temporary reduction on the B.t. plots in the 5-day collection. Budworm mortality caused by a nuclear polyhedrosis virus was noted in only one prespray collection and in the postspray samples from the check plots; virus mortality rates never exceeded 3 percent (table 5). B.t. mortality was about 3 times greater at 5 days in the Thuricide treatments than Dipel, similar to the pattern of activity shown in the bioassay on 2-hour foliage samples. There is a possibility that the Dipel acted more rapidly and a high proportion of infected budworm had dropped off the trees before the 5-day sample.

Defoliation Estimates

In addition to rating the amount of feeding damage to current growth during the year of treatment, we rated feeding damage for the previous year (1979). Substantial feeding damage occurred in the test area during 1979, with defoliation levels on plots ranging from 50 to 75 percent (table 6). Defoliation increased about 30 percent on the check plots in 1980. In contrast, feeding levels were slightly lower in all three B.t. treatments. The 1980 data were subjected to an analysis of covariance, using the prespray budworm density as a covariate. Sheffe's test of the adjusted means showed that defoliation was significantly lower for the two Thuricide treatments than the check, but at $P \leq 10\%$. It is reasonable to assume that foliage protection was adversely affected by the delay in spraying.

CONCLUSIONS

1. All of the treatments with Bacillus thuringiensis provided significant reductions in budworm populations and averaged about 90 percent (uncorrected). However, the results were weakened by an 80-percent loss in population in the check.

2. Parasite activity was not affected by the treatments.

3. Although we were able to measure a reduction in defoliation, protection was minimized by the delay in spray applications.

4. Rainfastness of spray deposits was lower on the plots treated with Thuricide-32B in 25 percent molasses.

5. B.t. mortality rates in a foliage bioassay declined rapidly over time. Less than 50 percent of the original activity remained on the foliage at 3 days after spraying.

COOPERATION AND COORDINATION

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APPENDIX

Table 1.--Population density of western spruce budworm at various periods after aerial applications of *Bacillus thuringiensis*.

TREATMENT	NUMBER OF SBW/0.64 m^2 FOLIAGE				
	PRESPRAY	5-DAY	12-DAY	19-DAY	26-DAY
T-16B	202.1	71.4	63.4	29.3	20.3
T-32B	158.6	60.3	37.9	15.4	9.5
DIPEL 4L	200.2	79.1	49.7	25.3	19.5
CHECK	185.7	100.8	94.7	51.6	34.7

Figure 1.--Survival curves for western spruce budworm populations following aerial applications of *Bacillus thuringiensis*.

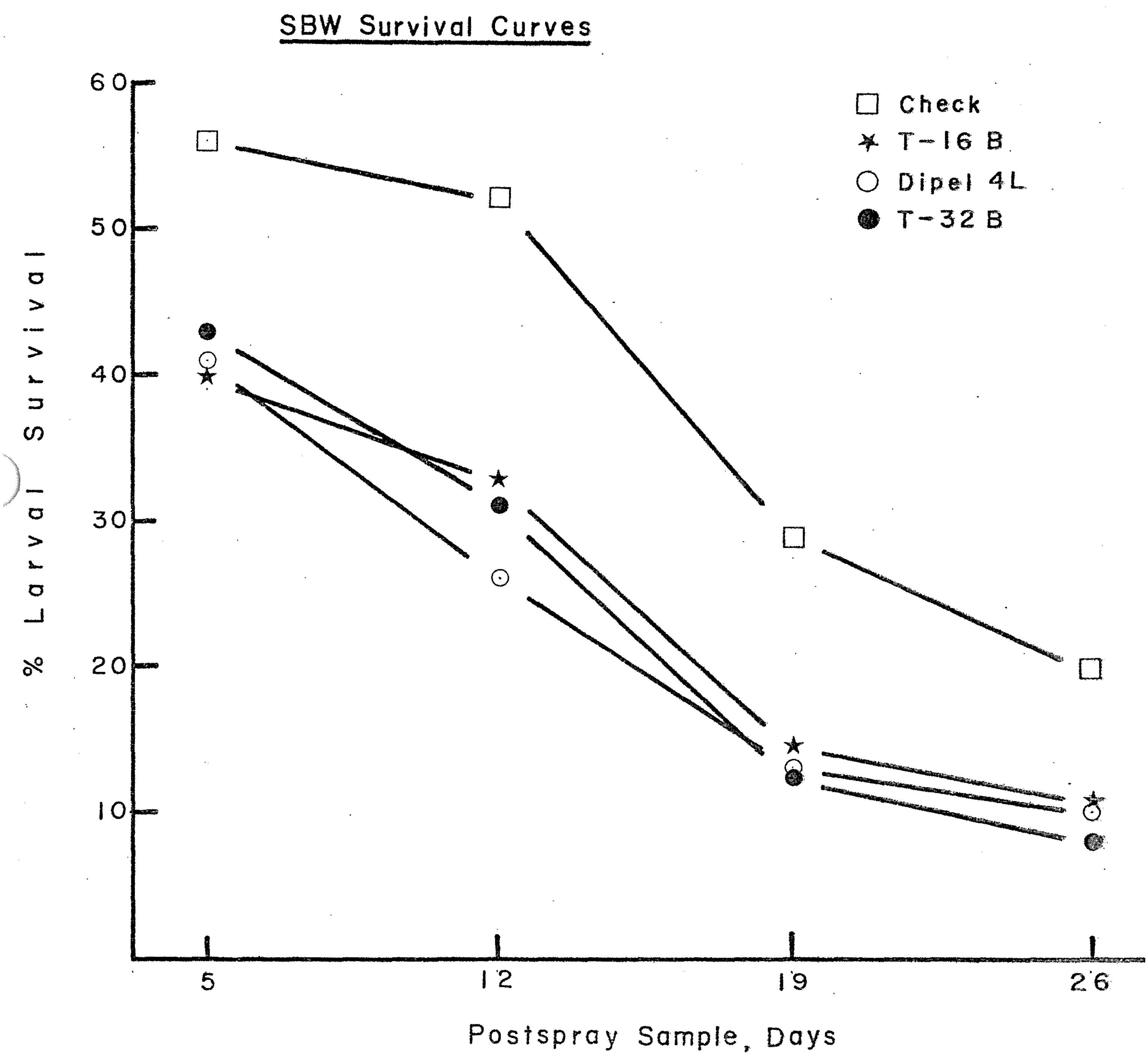
SBW Survival Curves
Check
T-16 B
Dipel 4L
T-32 B
% Larval Survival
60
50
40
30
20
10
5
12
19
26
Postspray Sample, Days

Table 2.--Residual activity of spray deposits on Douglas-fir foliage following aerial applications of <u>Bacillus thuringiensis</u>.

Postspray SAMPLE Timing	PERCENT Bt MORTALITY			
	T-16B	T-32B	DIPEL	~~AVERAGE~~
2 HR	36.5	40.8	19.1	~~32.1~~
3-DAY	19.3	8.2	10.4	~~12.6~~
5-DAY	0.7	0	1.3	~~0.7~~

Table 3.--Volume median diameter (VMD) and drop density (drops/cm^2) on Kromekote cards.

TREATMENT	VMD	DROPS/cm^2
THURICIDE-16B	149.5	15.9
THURICIDE-32B	152.8	14.3
DIPEL 4L	121.3	7.3

Table 4.--Percent parasitism of western spruce budworm at various periods after aerial applications of _Bacillus thuringiensis_.

SAMPLE	PERCENT PARASITISM				
TIMING	N	T-16B	T-32B	DIPEL 4L	CHECK
PRESPRAY	2135	41.9	41.7	42.2	37.0
5-DAY	2375	26.7	25.6	34.4	41.5
12-DAY	2452	36.2	37.3	34.7	34.6
19-DAY	1239	27.8	21.3	28.6	25.5
26-DAY	1443	15.9	3.9	9.3	9.6

Table 5.--Prevalence of disease mortality in western spruce budworm at various periods after aerial applications of _Bacillus thuringiensis_.

SAMPLE	PERCENT DISEASED LARVAE				
TIMING	N	T-16B	T-32B	DIPEL 4L	CHECK
PRESPRAY	2135	0.5	0	0	0
5-DAY	2375	21.3	25.0	7.8	2.8
12-DAY	2452	5.9	8.0	1.1	1.4
19-DAY	1239	7.4	5.6	1.1	0.7
26-DAY	1443	1.0	0.3	1.6	0.6

Table 6.--Percent defoliation by western spruce budworm in the year prior to (1979) and during the year of treatment (1980) with aerial applications of Bacillus thuringiensis.

TREATMENT	PERCENT DEFOLIATION 1979	1980
T-16B	68.9	62.9[a/]
T-32B	65.5	62.8
DIPEL 4L	75.5	65.6
CHECK	50.0	81.9

a/ Means adjusted to a common level using prespray budworm larval density/0.64 m^2 of foliage as a co-variate.